

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraphs at page 3, line 24 - page 4, line 12 with the following amended paragraphs:

-- ~~Fig. 1 illustrates~~ Figs. 1A-1E illustrate the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

~~Fig. 2 illustrates~~ Figs. 2A-2E illustrate the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

~~Fig. 3 illustrates~~ Figs. 3A-3E illustrate the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

~~Fig. 4 illustrates~~ Figs. 4A-4E illustrate the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

~~Fig. 5 illustrates~~ Figs. 5A-5E illustrate the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

~~Fig. 6 illustrates~~ Figs. 6A-6E illustrate the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

~~Fig. 7 illustrates~~ Figs. 7A-7E illustrate the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

~~Fig. 8 illustrates~~ Figs. 8A-8D illustrate the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

~~Fig. 9 illustrates~~ Figs. 9A-9E illustrate the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*. --

Please replace the paragraph beginning at page 5, line 14, with the following amended paragraph:

-- Some of the nucleotides in the contigs had been previously released. (~~See [ftp](http://ftp.tigr.org/pub/data/n_meningitidis)~~ available at [11ftp.tigr.org/pub/data/n_meningitidis](http://ftp.tigr.org/pub/data/n_meningitidis) on the world-wide web or “WWW”). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i. d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the “NMB” name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the “NMB open reading frames”. --

Please replace the paragraph beginning at page 8, line 24, with the following amended paragraph:

-- Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (~~http://~~ available at www.ncbi.nlm.nih.gov) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul et al. (1997) Gapped BLAST and PSI- BLAST: new generation of protein database search programs. Nucleic Acids Research 25: 2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB, SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein. --

Please replace the paragraph beginning at page 9, line 9, with the following amended paragraph:

-- Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (available at <http://www.psort.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE). --

Please replace the paragraphs beginning at page 58, line 8 and ending at page 59, line 27 (the section entitled "Oligonucleotide design") with the following amended paragraphs:

-- Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5'primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail: CGCGGATCCCATATG (*Bam*HI-*Nde*I) SEQ ID NO: 108

CGCGGATCCGCTAGC (*Bam*HI-*Nhe*I) SEQ ID NO: 109

CCGGAATTCTAGCTAGC (*Eco*RI-*Nhe*I) SEQ ID NO: 110

3'-end primer tail: CCCGCTCGAG (*Xho*I) SEQ ID NO: 111

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5'primers were used for each ORF; the same 3' *XhoI* primer was used as before:

5'-end primer tail: GGAATTCCCATATGGCCATGG (*NdeI*) SEQ ID NO: 112

5'-end primer tail: CGGGATCC (*BamHI*) SEQ ID NO: 113

Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*) SEQ ID NO: 114

3'-end primer tail: CGGGATCC (*BamHI*) SEQ ID NO: 115

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml NH₄-OH, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1 ml of water. OD₂₆₀ was determined

using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/ μ l.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons maybe changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC. –

Please add the following paragraph to the end of the specification:

-- This application hereby incorporates sequence listing in the compact disc formatted from an IBM-PC Compatible computer, compatible with MS-Windows. The compact disc contains the following file: SEQLIST223002100400.txt, containing 2.85 MB. This file was created on November 22, 2005. --